

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A probe for detection and quantification of a lipid second messenger, which comprises:

a polypeptide which can specifically bind the lipid second messenger,
a first chromophore linked to one end of the polypeptide through a rigid linker sequence;
a second chromophore linked to another end of the polypeptide through a second rigid linker sequence, wherein the second chromophore has a different fluorescence wavelength from the first chromophore, and ~~the second rigid linker sequence has a flexible site acting as a hinge~~
the second linker sequence is rigid except for a single flexible site acting as a hinge; and
a membrane localization sequence linked to the second chromophore through a third rigid linker sequence, wherein when the polypeptide is bound to the lipid second messenger, the first and second chromophores are capable of Fluorescence Resonance Energy Transfer (FRET).

2. (Previously Presented) The probe for detection and quantification of a lipid second messenger of claim 1, wherein the polypeptide which can specifically bind the lipid second messenger is a lipid second messenger-binding protein.

3. (Previously Presented) The probe for detection and quantification of a lipid second messenger of claim 2, wherein the lipid second messenger-binding protein is a pleckstrin homology domain from General Receptor for Phosphoinositides-1 (GRP1).

4. (Previously Presented) The probe for detection and quantification of a lipid second messenger of claim 1, wherein the chromophores are a cyan fluorescent protein linked to N-terminal end of the polypeptide and a yellow fluorescent protein linked to C-terminal end of the polypeptide.

5. (Previously Presented) The probe for detection and quantification of a lipid second messenger of claim 1, wherein the first, second and third linker sequences comprise a rigid α -helix linker consisting of repeated sequences of SEQ ID NO: 1.

6. (Currently Amended) The probe for detection and quantification of a lipid second messenger of claim 1, wherein the single flexible site of the second linker sequence is at least one linker sequence has a single di-glycine motif.

7. (Previously Presented) The probe for detection and quantification of a lipid second messenger of claim 1, wherein the membrane localization sequence is a lipidized sequence or a transmembrane sequence.

8. (Withdrawn) A method for detecting and quantifying a lipid second messenger, which comprises:

co-existing the probe for detection and quantification of a lipid second messenger of claim 1 with the lipid second messenger; and
measuring changes in fluorescence spectra.

9. (Withdrawn) The method for detecting and quantifying a lipid second messenger according to claim 8, which comprises:

introducing a polynucleotide to express the probe for detection and quantification of a lipid second messenger into cells; and
co-existing the probe with the lipid second messenger.

10. (Withdrawn) The method for detecting and quantifying a lipid second messenger according to claim 8, which comprises:

introducing a polynucleotide to express the probe for detection and quantification of a lipid second messenger into a non-human totipotent cell; and
ontogenizing the cell to non-human animal, thereby co-existing the probe with the lipid second messenger in all cells of the animal or offspring animal.

11. (Withdrawn) The method for detecting and quantifying a lipid second messenger according to claim 9, wherein the probe for detection and quantification of a lipid second messenger is fixed on membrane in the cells, and the lipid second messenger produced in the membrane is detected and quantified.

- 12. (Withdrawn)** A non-human animal or offspring animal thereof, which is obtained by:
introducing a polynucleotide to express the probe for detection and quantification of a lipid second messenger of claim 1 into a non-human totipotent cell; and
ontogenizing the cell to the non-human animal.
- 13. (Withdrawn)** A method for screening a substance for quantifying a lipid second messenger, in the cells of the non-human animal or offspring animal thereof of claim 12, which comprise introducing a test sample into the non-human animal or the offspring animal thereof.
- 14. (Withdrawn)** The method for detecting and quantifying a lipid second messenger according to claim 10, wherein the probe for detection and quantification of a lipid second messenger is fixed on membrane in the cells, and the lipid second messenger produced in the membrane is detected and quantified.